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Iowa State College of Agriculture and
Mechanic Arts

TEMPERATURE AND THE GROWTH OF YEAST

A Dissertation

Submitted to the Graduate
Faculty in Candidacy for the
Degree of

Doctor of Philosophy

by

Frank F. Sherwood

Approved:

Signature was redacted for privacy.

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1924

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TEMPERATURE AND THE GROWTH OF YEAST

I. Introduction.

In carrying on investigations dealing with vital phenomena we must keep in mind the fact that we are dealing with very complex systems. If we attempt to analyze living matter by separating its chemical constituents and identifying them we find that we are no longer dealing with living matter because the reagents which we employ immediately and abruptly suspend its characteristic functions. For example, we may make an analysis of yeast and obtain some interesting products but we no longer have yeast cells and the products we obtain, although very interesting in themselves, may bear only a remote relationship to those which were present in the living organism. This method of investigation has been employed by numerous investigators in an attempt to arrive at or determine the nutritional requirements of yeast. That is, they would analyze a sample of yeast and assume that all the constituents found were absolutely necessary for the growth of yeast and consequently an artificial medium should contain all the constituents found. This is, of course, false reasoning as the mere presence of any given element is not an a priori reason for believing that this particular element is absolutely essential for the normal growth and reproduction of yeast.

A second method of study consists in an analysis of the chemical phenomena which accompany or underlie the activities of living, undisturbed and more or less functioning protoplasm.

As Robertson¹ says, "In the so-called 'exact sciences', namely mechanics and physics, we have, as a rule the power to isolate more or less completely any phenomenon or group of phenomena which we wish to study, and to guard them from disturbance by the intrusion of accidental variables. For example, it is not a difficult matter to demonstrate that a falling body experiences a constant acceleration, the most serious intrusive variable being the friction of the air, a variable which can now be very readily excluded in a variety of ways. Similarly, in chemistry, it is not a difficult matter to observe the progress and equilibrium of such a reaction, as, for example, the reduction of iron oxide by hydrogen. However, when we come to organic chemistry we encounter much more complex phenomena and when we take up the study of life processes we find the difficulties which are encountered in studying organic reactions in laboratory glass ware are enormously magnified in studying reactions which occur in living matter."

In making a study of life processes two methods have been employed, first, the influence of temperature upon life processes and, second, the growth rate. Before going fur-

ther it will, perhaps, be well to give a brief summary of the work that has been done regarding the influence of temperature on chemical reactions:

II. The Influence of Temperature on Rates of Reaction.

Due to the fact that the influence of temperature on chemical reactions is so pronounced it has been quite generally recognized as one of the most important factors in the study of chemical changes. Although many interesting facts have been brought to light and many phenomena explained there is still much to be learned concerning the influence of temperature upon chemical reactions.

The velocities of most purely chemical reactions increase rapidly with rise of temperature. For example, the inversion of cane sugar proceeds five times as fast at 55 degrees as it does at 25 degrees; the conversion of solid Ammonium Cyanate into Urea is fifty times as rapid at 57 degrees as it is at 33 degrees; the transformation of dibromosuccinic acid into bromomaleic acid goes three thousand times as rapidly at 101 degrees as at 15 degrees; and although the reaction between hydrogen and oxygen is so slow at 155 degrees that no sign of combination can be detected after many months, yet at about 600 degrees the combination takes place with explosive violence. Dewar, too, has shown that at the temperature of liquid air (-183 degrees) photographic action

is 20 percent; and at the temperature of liquid hydrogen (-250 degrees) it is but 10 percent of its value at ordinary temperature.

The first important work carried out upon reactions in homogenous systems was that of Wilhelmy² who measured the rate of the inversion of cane sugar in aqueous solution into dextrose and laevulose under the catalytic action of a small quantity of acid (hydrochloric or nitric). Using a polarimeter, Wilhelmy determined the rate of reaction from the change in the angle of rotation at different time intervals. Wilhelmy made the assumption that the rate at which the inversion took place, is proportional to the quantity of cane sugar still uninverted in the solution. This important assumption of Wilhelmy is the basis of the law of mass action. If the time be expressed by t and the quantity of sugar inverted after any given time be x , the velocity of inversion is $\frac{dx}{dt}$. This quantity $\frac{dx}{dt}$ is, according to Wilhelmy, proportional to the quantity of cane sugar remaining, which may be represented by $(a-x)$, where (a) is the original quantity of cane sugar when t equals 0. Hence, Wilhelmy's equation is -- $\frac{dx}{dt} = k(a-x)$, where k is a proportionality factor called the "velocity constant" of the reaction. On integration one obtains,

$$k = \frac{1}{t} \log \frac{a}{a-x} \text{ or } k_1 = \frac{1}{t} \log_{10} \frac{a}{a-x} \quad (1)$$

where $k = 2.303 k_1$.

Wilhelmy was the first to construct the differential equation quoted above for the calculation of a chemical reaction velocity. Using nitric acid as the catalyst (at 15 degrees) he succeeded in verifying the above experimentally.

In the above equation k is the specific velocity of the reaction, constant, provided we keep the temperature constant. If the temperature changes during the reaction, k is no longer constant, but increases proportionally with the temperature. For example, Harcourt and Esson³ found that in the reaction -- H_2O_2 plus $2\text{HI} = \text{I}_2$ plus $2\text{H}_2\text{O}$ k varied with the temperature, as indicated by the following numbers:

When t equals 5, 10, 20, 30, 40, 50,

k equals 1.00, 2.08, 4.32, 8.38, 16, 19, 30, 95.

Various formulae have been proposed, since Wilhelmy proposed his, by Berthelot, Harcourt and Essen, Warder, Urech, Hood, Vant Hoff, and Arrhenius.

In dealing with the influence of temperature on chemical equilibria if we let A and B represent two different reacting substances at the point of equilibrium, the velocities of the opposing reactions are the same, or--

$$k_1 C_A = k_2 C_B; \text{ therefore, } K = \frac{k_1}{k_2} = \frac{C_B}{C_A} \quad (2)$$

Since this relation holds good only when the temperature is constant, Nernst proposes to call K the "reaction isotherm". In his work, *Etudes de dynamique chimie*, Vant Hoff

has deduced the expression--

$$\frac{d \log k_1}{dT} - \frac{d \log k_2}{dT} = \frac{q}{2T^2} \quad (3)$$

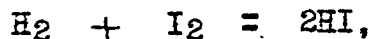
from the mechanical theory of heat for the relation between k_1 and k_2 and the quantity (q) of heat set free when one gram molecule of A is transformed into B at the absolute temperature T.

"Although this equation, says vant Hoff, does not directly express the relation between the velocity constants of the two inverse reactions and the temperature, yet it does show that this relation must have the form --

$$\frac{d \log K}{dT} = \frac{P}{T^2} \text{ plus } Q \quad (4)$$

where P and Q are constants." The differential coefficient on the left side of (4) refers to the variation of the value of K with temperature. This law of chemical equilibrium, it will be observed, deals only with the end state of a reaction, and it has nothing to say about the time in which that end state will be attained. Although thermodynamics gives us a relation between the state of equilibrium and the thermal value of a reaction, the time factor finds no place in that expression. P is not necessarily constant because the quantity of heat (q) absorbed or evolved in any reaction changes with the temperature. This change, however, is usually so small that we may often assume that P is really constant throughout a small interval of temperature, but the thermal value of some reactions varies considerably

with temperature. For example, the thermal value of the reaction,



at 10 degrees is-- 6100 cal.; at 180 degrees 1885 cal., and at 520 degrees 4444 cal.

P is really a function of temperature and for this reason van't Hoff's solution of the problem is still indefinite, owing to the lack of any information as to the form of the function--

$$q = f(T) \text{ ----- (5)}$$

Whenever we meet with a case like this it is the usual thing to write--

$$f(T) = A + BT + CT^2 + DT^3 + \text{-- (6)}$$

where A, B, C, -- are constants, because we know that whenever a physical change is represented by such an expression we can generally approximate very close to the numerical value of (q) by increasing the number of terms included in the calculation.

Practically all the empirical formulae which have been proposed by different investigators to represent the unknown relation between the temperature and the velocity of a chemical reaction can be referred to the formula--

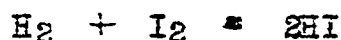
$$\frac{d \log K}{dT} = \frac{A + \frac{BT}{T^2} + CT^2}{T^2} \text{ ---- (7)}$$

which on intergration assumes the form--

$$\log.K = \frac{A}{T} + B \log. T + CT + \dots + \text{constant (8)}$$

Where A, B, and C are constants.

The first three terms of the series (6) were employed by C. Hahn⁵ to represent the influence of temperature upon the reaction between hydrogen and carbon dioxide at high temperatures; and by M. Bodenstein⁶ to represent the influence of temperature on K in the reaction--



D. M. Kocij omitted all terms succeeding the second, and thus obtained the expression--

$$\frac{d \log.K}{d T} = \frac{A}{T^2}; \text{ or, } \log K = \frac{A}{T} + B \log T + \text{a constant (9)}$$

for the influence of temperature on the decomposition of phosphine and arsine.

Arrhenius⁸ only retained the first term of the series (7) and employed the equation--

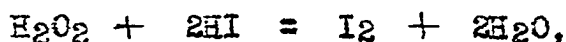
$$\frac{d \log K}{d T} = \frac{A}{T^2}; \text{ or, } \log k_1 - \log k_0 = A \left(\frac{1}{T_1} - \frac{1}{T_0} \right) \quad (10)$$

$$\text{i.e., } k = k_0 e^{\frac{A(T_0 - T_1)}{2(T_1 T_0)}}$$

The above equation has given fairly satisfactory results with a large number of measurements to which it has been applied. Arrhenius himself compared it with measurements by Hood⁹, Warder¹⁰, Schwab and Hecht¹¹ and Conrad, as well as with the experiments of Urach¹² and of Spchr¹³ on the inversion of cane sugar. Price¹⁴ employed it in his work on the hydrolysis of the esters; I. Remsen¹⁵ and E. E. Reid for the hydrolysis of

nitrobenzamide by the bases; J. H. Kastle¹⁶ and A. S. Loevenhart for the oxidation of formaldehyde by hydrogen peroxide; Goldschmidt¹⁷ and Reinders for the conversion of diazo amido into amido diazo compounds; Ley¹⁸ also obtained very fair results with the transformation of anisynaldoxime acetate into the corresponding nitrile, and for the intramolecular transformation of the acetates of anisynaldoxime, p-chlorbenzsynaldoxime, and thiophensynaldoxime.

Harcourt and Essen¹⁸, in their study of the reaction--



sought the form of the function (T). They found that if temperature be designated on the absolute scale, and k_0 be the value of k at T_0 , and k the value of the velocity at some other temperature, T , then--

$$\frac{k}{k_0} = \frac{(T)^B}{(T_0)^B}, \quad (11)$$

where B is a constant to be evaluated from the experimental data.

Harcourt and Esson used the equation--

$$\frac{d \log K}{d T} = \frac{B}{T}, \quad (12)$$

which is a special case of the fundamental equation (7) which on intergration, assumes the form indicated in equation (11).

van t Hoff's equation--

$$\frac{d \log K}{d T} = \frac{A}{T} + C \quad (13)$$

or, integrated--

$$\log K = \frac{A}{T} + CT + \text{a constant} \quad (14)$$

is a special case of the fundamental equation obtained by putting $B = 0$. Schwab¹⁹ applied this equation to the transformation of dibromosuccinic acid into bromo maleic acid, and to the reaction between sodium mon chloracetate and sodium hydroxide; J. Spohr²⁰ to the inversion of cane sugar; and G. Buchbock used it to represent his results on the influence of temperature on the decomposition of carbonyl sulphide by water.

If we neglect the first two terms of the series in the original equation we get--

$$\frac{d \log K}{dT} = C, \quad (15)$$

which on integration becomes--

$$\log K = CT + \text{a constant.}$$

If natural logarithms are employed this may be written--

$$k = k_0 e^{CT} \quad (16).$$

The above formula was employed by Pendlebury²² and Seward in their study of the interaction of hydrogen iodide; by Tamman²³ to represent the velocity of crystallization at different temperatures; by Reid²⁴ to represent the hydrolysis of nitrobenzamide; and by Veley²⁵ for the reaction between nitric acid and copper.

If common logarithms be employed, expression (16) becomes--

$$k = k_0 \times 10^{CT}, \quad (17)$$

which was used by Bugarszky to represent the influence of temperature on the reaction between bromine and ethyl alcohol; and by Hecht²⁷ and Conrad in their work on the action of alkyl iodides on sodium alkylates.

If we take the logarithms to some other base, say a , we get--

$$k = k_0 a^{CT} \quad (18).$$

The above equation in a modified form was used by Berthelot in 1862, for the action of acetic acid upon ethyl alcohol; by Spring²⁹ for the dissolution of marble in mineral acids and by Hood³⁰ for the rate of oxidation of ferrous sulphate by potassium chromate.

Arrhenius maintains the view that the increase of velocity of a chemical reaction with temperature cannot be explained by any change in the physical properties of the solution with temperature. He proposes the hypothesis that cane sugar contains two kinds of molecules--active and passive. The active can alone be hydrolyzed by the acid, while the passive molecules are not acted upon. The amount of "active" cane sugar in solution is supposed to be very small in comparison with the "inactive" sugar. In order to explain the influence of temperature on the rate of inversion, Arrhenius still further assumes that the quantity of active cane sugar must increase very rapidly,--about 12 percent for each degree rise of temperature--and this at the cost of the inactive sugar. The transformation of inactive into active sugar is said to be due either to a rearrangement of the atoms or to the introduction of water into the molecule of inactive sugar. A state of equilibrium between the active and inactive molecules of cane sugar will be attained when the respective concentrations $C_a =$

C_1 ; and from van't Hoff's equation (3).

$$\frac{d \log K}{dT} = \frac{q}{2T^2}; K_1 = K_0 e^{\frac{q}{2} \left(\frac{T_1 - T_0}{T_1 T_0} \right)}, \quad (19)$$

where q denotes the thermal value of the transformation of inactive into active cane sugar, and K_1 and K_0 are the equilibrium constants at the two different temperatures.

If the velocities of the reactions at the two temperatures be V_1 and V_0 , then it is supposed that--
 $V_1 = V_0 e^{\frac{q(T_1 - T_0)}{2(T_1 T_0)}}$; or q is about 25,600 calories per gram molecule of inactive sugar.

Robertson says that, "the behavior of physical phenomena which are affected by the temperature, is quite different. That is, the effect of temperature is in these phenomena qualitatively much less than it is in phenomena which arise from chemical transformations, and for this reason we may, with a fair degree of confidence, employ the temperature coefficient of a complex phenomenon which involves physical as well as chemical changes as a means of gauging the extent to which the velocity of the process is governed by the chemical transformations which it involves. If the pace is set by the rate at which some chemical change transpires, then the rapidity of the process will be at least doubled and not improbably, more than doubled by a rise of 10 degrees in temperature. But if the chemical transformations are subordinate to some physical process and must await its development before they can proceed, or if they are simply consequent upon physical changes

the pace of the whole process will be set by this physical event and the temperature coefficient of the process may be expected to be less than two or even very considerably less than two."

Bayliss³² does not agree with the above statements and believes that caution must be exercised in our conclusions as to whether a process is of a physical or chemical nature on the basis of observed temperature coefficients. His views are supported by the fact that a goodly number of chemical reactions exhibit a low value for temperature coefficients while some are even retarded by a rise in temperature.

For example, Benson³³ found that the rate of reaction in the case of the liberation of iodine from a mixture of KI, FeSO_4 and CrO_3 is less at 30 degrees than at 0 degrees while the rate of reduction of ferric sulphate by iron in acid solution appears to decrease with increase of temperature.

Treutz and Volkmann³⁴ found that the saponification of ethyl butyrate by barium hydroxide between 50 and 60 degrees has the low value for a chemical reaction of 1.33 for ten degrees whereas diffusion, a physical process, has a value nearly as high, viz., 1.28. Chick³⁵ and Martin find that the heat coagulation of haemoglobin has the extraordinarily high temperature coefficient of 13.8 for ten degrees, while that of albumin is even higher.

P. von Schroeder³⁶ finds that gelatine solutions, in a particular condition, have a viscosity at 21 degrees repre-

sented by 15.76, whereas at 31 degrees it is only 1.42. This is interesting due to the fact that colloids of the type of gelatine play a large part in vital processes.

We find that even in a simple uncomplicated chemical transformation the temperature coefficient for ten degrees temperature intervall is not constant, for, reverting to the equation--

$$\frac{K_1}{K_0} = e^{\frac{q}{2} \left(\frac{T_1 - T_0}{T_1 T_0} \right)} \quad (20)$$

we see that the temperature coefficient for 10 degrees is given by-- $\frac{K_1}{K_0} = e^{\frac{q}{2} \left(\frac{10}{T_1 T_0} \right)}$ it is therefore not independent of the temperature employed; in fact the temperature coefficient must invariably decrease as the temperature rises. Assuming a value of $q = (13,200)$ which would yield a coefficient of 2 between the temperatures of 30 and 40 degrees, the following table shows the coefficients which might be anticipated at other temperatures:

Temperature Interval.	Temperature Coefficient.
0-----10 degrees	2.34
10-----20 "	2.22
20-----30 "	2.11
30-----40 "	2.00
40-----50 "	1.92

III. The Influence of Temperature Upon the Growth of Yeast.

Temperature coefficients for yeast were determined by

Aberson³⁷ working between 12 and 33 degrees giving a mean temperature quotient for 10 degrees ($K_t \div 10/K_t$) = 2.72. Herzog³⁸ gives values of velocities from 14.5 to 28.5 degrees, with $K_{24.5}/K_{14.5} = 2.88$. Slator³⁹ confirmed these results, but showed that the temperature quotient varied with the temperature. He obtained his temperature quotients by growing yeast in a closed vessel connected with a manometer and took the time required to raise the column of mercury 4 mm. at temperatures ranging from 5 to 40 degrees. The results of his experiments are shown in the following table:

Temp.	Observed ratio	Percentage correction	V_{t+5}/V_t	V_{t+10}/V_t
5				
10	2.94	10	2.65	5.6
15	2.29	8	2.11	3.8
20	1.89	5	1.80	2.80
25	1.65	5	1.57	2.25
30	1.50	5	1.43	1.95
35	1.42	5	1.35	1.60
40	1.27	5	1.20	

Slator states that below five degrees the reaction proceeded too slowly to be measured while above forty degrees the enzyme was destroyed. However his experiments were carried out in a synthetic medium apparently made up on the basis of a chemical analysis of yeast and therefore probably not the best medium obtainable for his purpose. Furthermore, he did not consider the relation of temperature to the composition

of the medium. Since it has been shown, by Fulmer, Nelson and Sherwood⁴⁰, that there is an optimum concentration of the salts used that varies with the temperature, it is quite probable that he would have obtained entirely different results had he varied the composition of his medium with the temperature.

In a study of the growth rate of yeast A. Slator⁴¹, in 1913, used four methods of estimating K. The validity of these equations was tested by measuring the rate of growth of a pure culture of a Burton yeast in highly hopped wort of specific gravity 1.040. It was found that K, the constant of unrestricted growth of this culture, could be determined by methods of yeast counting and also by measuring the rate of fermentation of the growing yeast cells. Slator also showed that until the yeast crop reached about ten million cells per cubic centimeter (a count of 40) the rate of increase at any moment is proportional to the number of cells then present; whence $k = \frac{\log C}{t}$.

About the same time T. Carlson⁴² proposed a more complicated relation between C and t from the assumption that, besides being proportional to the number of cells present, the rate is also proportional to the square of the concentration of the foodstuff remaining available in the wort.

Clark⁴³ showed that under the conditions specified in his work, that the rate of reproduction is independent of the concentration of alcohol until this reaches 1.8 grams per 100 c.c.

In 1914 Horace T. Brown⁴⁴ determined the rate of reproduction of yeast when grown in mixtures of air-saturated wort, with

wort from which all air had been removed by boiling in an atmosphere of hydrogen.

Fulmer⁴⁵ measured the rate of reproduction of yeast in wort with and without ammonium fluoride and showed that the cells which are not killed outright undergo a period of paralysis, after which they reproduce, giving rise to fluoride-resistant cells.

As stated above in no case where thermal coefficients of growth rates have been determined have the investigators taken into consideration the composition of their medium. Reaction rates in solution are usually appreciably influenced by the presence of neutral salts which apparently have nothing to do with the reaction itself. In many cases this "neutral salt effect", as it is called, may be interpreted as due, at least partially, to the influence of the ions of the salt upon the thermodynamic environment prevailing within the solution, for the rate of any given reaction is very powerfully influenced by the nature of the medium in which it takes place.

In determining growth rates or thermal coefficients it is therefore essential that the experiments be conducted as far as possible under comparable conditions as regards thermodynamic environment, since the logarithmic rate of reproduction may be influenced by changes in the reaction of the medium, in surface tension, in osmotic pressure, in pressure, in temperature, in permeability and in the state of hydration of cell proteins.

In a previous communication, Fulmer⁴⁶, Nelson and Sherwood made a study, among other things, of the effect of ammonium salts upon the growth of yeast in synthetic media. The findings of the above authors may be summarized as follows:

1. There is an optimum concentration of ammonium salts for the growth of yeast at a given temperature.
2. For five ammonium salts tested the optimum concentration for the growth of yeast was found to be at the same normality, that is, the effect is due to the ammonium ion.
3. The higher the temperature the greater the concentration of ammonium salt required for optimum reproduction.
4. At all temperatures studied (21, 30, 35, and 40 degrees) the concentration of ammonium salt for the optimum growth of yeast was found to coincide with the concentration of the salt in which a protein, wheat gluten, was the least swollen.

IV. Statement of Problem.

The object of the work presented by the author is to determine growth rates and temperature coefficients by adhering to the following:

1. Determine the best possible medium for each desired temperature.
2. After determining the best medium for each temperature, determine the growth rates and from these values calculate the temperature coefficients for the various time intervals.

V. Determination of the Optimum Medium for Various Temperatures.

Method: The method employed was identical with the method outlined in the preceding paper⁴⁹ except in the case of the determination of growth rates the flasks were gently rocked in a small rocker. James O'Sullivan⁵⁰ shows that agitation is absolutely necessary in making a quantitative study of the growth rates of yeast. Clark⁴³ also used this method in his work.

It was found that there is an optimum concentration of ammonium chloride for the growth of yeast at the following temperatures: 0, 10, 20, 25, 30, 35, 40, 42 and 43 degrees.

The results of the above investigations are shown for the different temperatures in Tables 1 to 9 inclusive and summarized in Table 10.

It was found that the concentration of ammonium salt for the optimum growth of yeast coincides with the concentration of the salt in which gluten is the least swollen⁴⁰. It will be noticed that the best growths were obtained at 40 degrees. At 42 degrees the yeast were growing quite well while at 43 degrees there is a rather sharp break, the total crop for medium E being a count of 3.4 and for wort 3.8 at the end of 68 hours, as compared with 186 for medium E and 630 for wort at 42° C.

TABLE I.

Growth of Yeast at 0° C. Varying NH_4Cl .

Normality of NH_4Cl	I = 1 Count 24 hrs.	I = 1 Count 48 hrs.	I = 1 Count 96 hrs.
0.000	2.3	2.2	2.1
0.0118	2.6*	3.6*	3.8*
0.01475	1.8	2.4	2.3
0.02360	1.3	1.1	1.1
0.0295	1.2	.6	.9
0.0354	1.0	.5	.8
0.03835	1.0		
0.0413	.5		
0.0472	.4		
0.0590	.4		

TABLE II.

Growth of Yeast at 10° C. Varying NH_4Cl .

Normality of NH_4Cl	:	I = 1 Count 37 hrs.	:	I = 1 Count 45 hrs.
0.000		11.3		15
0.0118		19.6		21
0.02065		24.0		28
0.02360		31.50		39
0.02655		27.0		29.5
0.0295		19.0		22
0.0354		13.5		17
0.0413		10		12

TABLE III.

Growth of Yeast at 20° C. Varying NH_4Cl .

Normality:	I = 1	I = 1	I = 1	I = 1	I = 1	I = 1	I = 1
of	Count	Count	Count	Count	Count	Count	Count
NH_4Cl	24 hr.	30 hr.	30 hr.	36 hr.	24 hr.	46 hr.	46 hr.
0.000	43	26	45	45	54	67	89
0.0118	47	58	87	103	96	113	120
0.0236	53	92	103	109	108	166	143
0.0295	55	101	158	158	150	224	168
0.03009		39	77	83	47	101	104
0.03068		31	65	53	45	84	73
0.03127		50	66	42	47	66	73
0.03186		27		43		69	76
0.0324	50		61	40	46	67	73
0.0354	32			38		66	
0.03835	30	28	42	39	49		76
0.0472	27	28	42	45	49	68	73
0.0590	22						

TABLE IV.

Growth of Yeast at 25° C. Varying NH_4Cl .

Normality of NH_4Cl	:	I = 1 Count 24 hr.	:	I = 1 Count 36 hr.
0.000	:	30	:	45
0.0118	:	40	:	56
0.02360	:	48	:	98
0.0295	:	78	:	103
0.03068	:	96	:	109
0.03186	:	122	:	156
0.0324	:	80	:	89
0.0354	:	60	:	69
0.0413	:	49	:	56
0.0472	:	45	:	49

TABLE V.

Growth of Yeast at 30° C. Varying NH_4Cl .

Normality of NH_4Cl	I = 0.5 Count 48 hr.	I = 0.5 Count 83 hr.	I = 1 Count 24 hr.	I = 1 Count 48 hr.
0.000	53	46	56	80
0.0118	194	242	102	212
0.02360	256	280	115	260
0.02655	274	294	117	273
0.0295	295	312	138	280
0.0324	306	313	156	296
0.3422✓	356	335	174	311
0.0354	389	369	184	354
0.03658	270	283	120	276
0.03835	220	229	96	201
0.0413	213	226	85	161
0.0472	190	182	74	144
0.0590	173	177	67	140

TABLE VI.

Growth of Yeast at 35° C. Varying NH_4Cl .

Normality of NH_4Cl	:	1 = 1 Count 72 hr.	:	1 = 1 Count 24 hr.
0.000	:	11.7	:	57
0.0118	:	99	:	108
0.02360	:	188	:	144
0.0295	:	201	:	196
0.0324	:	243	:	225
0.0354	:	277	:	257
0.03835	:	314	:	273
0.0413	:	186	:	202
0.0472	:	177	:	197
0.059	:	168	:	143

TABLE VII.

Growth of Yeast at 40° C. Varying NH_4Cl .

Normality of NH_4Cl	I = 1 Count 72 hr.	I = 1 Count 44 hr.	I = 1 Count 72 hr.	I = 1 Count 77 hr.
0.000	11.5	57	59	62
0.0118	98	204	122	146
0.02360	190		146	213
0.02655				
0.0295	206	222	199	236
0.0324			242	260
0.03422				
0.0354	244	267		
0.03658			303	291
0.03835	280	276	363	310
0.0413	318	288	378	368
0.0472	174	216	251	164
0.059	160	198	156	142

TABLE VIII.

Growth of Yeast at 42°. Varying NH_4Cl .

Normality of NH_4Cl	1 = 1 Count 24 hr.	1 = 1 Count 48 hr.	1 = 1 Count 60 hr.
0.000	30	75	18
0.02360	59	123	35
0.0295	76	149	47
0.0354	135	173	63
0.03835	196	229	77
0.0413	239	282	99
0.04248	279	342	139
0.04366	206	289	156
0.0472	159	240	145
0.0590	143	186	139

TABLE IX.

Growth of Yeast at 43°. Varying NH_4Cl .

Normality of NH_4Cl	I = 1 Count 24 hr.	I = 1 Count 34 hr.	I = 1 Count 45 hr.
0.0000	1.00	1.1	1.2
0.03835	2.5	2.7	2.9
0.0413	2.6	2.8	3.00
0.0424	2.7	3.00	3.1
0.04307	3.1	3.2	3.3
0.04366	2.8	2.9	3.1
0.04425	2.7	2.8	3.0
0.0472	2.6	2.6	3.1

TABLE X.

Summary of the Effect of Ammonium Chloride Upon the Growth of Yeast in Medium E.

Temp.:	Optimum:	Time:	Maximum:	Time:	Maximum:	Time:	Max.:	Time:	Max.
: NH ₄ Cl :	:	: Growth :	:	: Growth :	:	: Growth:	:	: Growth	:
0	0.0118	24hr.	2.6	48hr.	3.6	96hr.	3.8		
10	0.02360	37	31.5	45	39				
20	0.0295	24	55	30	101	36	158	46	224
25	0.03186	24	122	36	156				
30	0.0354	48	389	24	184	48	354		
35	0.03835	72	314	24	273				
40	0.0413	72	318	44	288	72	378	77	388
42	0.04248	24	279	48	342	30	139		
43	0.04307	45	3.3						

It will also be noticed that yeast grow at 0 degrees although rather slowly. The results of the above investigations show that yeast will grow over a rather wide range of temperature if the proper environment is provided for them.

1. The Effect of Ammonium Chloride upon the Growth of Yeast in Wort.

During the course of the above investigations it occurred that it might be well to make a similar study of the effect of ammonium chloride upon the growth of yeast in wort, which is commonly considered to be the best known medium for the growth of yeast.

A few preliminary experiments seemed to indicate that there were two maximal concentrations of ammonium chloride for the growth of yeast in wort. The influence of ammonium chloride upon the growth of yeast in wort was studied for 10, 20, 30, 40 and 42 degrees and in each case it was found that there are two maximal concentrations for the growth of yeast in wort. The results are shown in Table 11.

TABLE XI.
The Effect of Ammonium Chloride upon the Growth of Yeast in Wort.

Temperature : 10 :		: 20 :		: 30 :		: 40 :		: 42 :	
Normality: Count : of NH ₄ Cl : 36 hr.:		Normality: Count : of NH ₄ Cl : 30 hr.:		Normality: Count : of NH ₄ Cl : 22 hr.:		Normality: Count : of NH ₄ Cl : 42.5 hr.:		Normality: Count : of NH ₄ Cl : 25 hr.:	
0.000	185	0.000	210	0.000	230	0.000	680	0.000	29
0.0059	210	0.0118	240	0.0118	270	0.0236	795	0.0118	63
0.0088	222	0.01475	253	0.0236	352	0.0259	825	0.0236	94
0.0118	235	0.0177	265	0.0294	232	0.02714	843	0.0294	157
0.0129	248	0.0182	273	0.0341		0.0283	900	0.0353	83
0.0135	260	0.0188	340	0.0353	242	0.0295	720	0.0482	86
0.0141	321	0.0194	258	0.0470	245	0.0354	692	0.0470	83
0.0147	245	0.0200	245	0.0588	244	0.0590	700	0.0588	84
0.0177	239	0.0236	233	0.0820	246	0.0826	713	0.0700	77
0.0354	226	0.0354	225	0.106	281	0.1060	733	0.0820	72
0.0531	240	0.0590	220	0.118	235	0.1180	772	0.0940	81
0.0590	256	0.0708	239	0.129	233	0.1416	685	0.1230	142
0.0649	269	0.0767	242					0.140	111
0.0708	245	0.0826	253					0.153	84
0.0826	232	0.0826	261						
		0.0885	241						
		0.0896	236						

A study of Table 11 brings out the following points:

1. There are two maximal concentrations, as in the synthetic medium, of ammonium chloride which vary with temperature, for the growth of yeast.
2. Less ammonium chloride is required for the growth of yeast in wort than in a synthetic medium, showing that there is present in the wort something which plays a similar role to that of ammonium ion.

2. A Second Maximum of Ammonium Chloride for the Growth of Yeast in Medium E.

In view of the above findings it was thought advisable to try higher concentrations of ammonium chloride in medium E, to ascertain whether or not there was a second maxima of ammonium chloride for the growth of yeast in this medium. Higher concentrations of ammonium chloride were tried at 10, 20, 30, and 40 degrees. In every case there was found a second maximum. The results are shown in Table 12. It was also found that the concentration of ammonium chloride for the second maxima corresponds to a second minimum point of hydration of gluten.

F. Boas⁴⁷, working with a bottom yeast in a 15% dextrose solution and various concentrations of ammonium sulphate, found a zone of maximum carbon dioxide production in salt concentrations between 0.5 and 0.05N. He also found two minima regions from 1.5 to 1.0N and from 0.016N down.

Pringsheim⁴⁸ carried out a series of experiments to determine the influence of the concentration of nitrogenous food on fermentation, with three races of yeast, "Lagor" yeast, "Frohberg" yeast, and a wine yeast. Peptone, synthetic leucine, leucine from molasses, asparagine and ammonium sulfate were used as sources of nitrogen. With synthetic leucine, asparagine and ammonium sulfate a well marked optimum concentration was found with two minima regions. Pringsheim explains these phenomena on the basis of the action of the different nitrogen concentrations on the plasma membrane at times causing a thickening and slow intake of sugar and consequently a slower fermentation, at times a loosening, resulting in the reverse action.

3. The Effect of K_2HPO_4 Upon the Growth of Yeast in Medium E.

The effect of various concentrations of dipotassium phosphate upon the growth of yeast was tried at 25°, 30°, 35°, and 40° C. It was found that the addition of dipotassium to the medium increased the growth of yeast with increase of the salt up to a concentration of 0.00464 normal. Further addition showed no improvement. The results of these determinations are shown in Table 13.

4. The Effect of Calcium Chloride Upon the Growth of Yeast in Medium E.

The effect of various concentrations of calcium chloride upon the growth of yeast was tried at 25°, 30°, 35°, and 40° C. It was found that the addition of calcium chloride showed an improvement in the medium up to a concentration of 0.1800 normal. The addition of larger quantities showed no improvement over 0.18 or normal. The results are tabulated in Table 14.

5. The Effect of Calcium Carbonate Upon the Growth of Yeast in Medium E.

Various concentrations of calcium carbonate were tried in medium E at 25°, 30°, 35°, and 40° C. It was found that the addition of calcium carbonate showed an improvement of the medium with increase of calcium carbonate up to a concentration of 0.0160 normal. Further additions showed no improvement over 0.016 normal. The results are tabulated in Table 15.

TABLE XIII.

Growth of Yeast, Varying K_2HPO_4 .

25° C.		30° C.		35° C.		40° C.	
Normality:	I=1	I=1	I=1	I=1	I=1	I=1	I=1
of	Count	Count	Count	Count	Count	Count	Count
K_2HPO_4	:30 hr.:	:66 hr.:	:42 hr.:	:24 hr.:		:24 hr.:	:72 hr.:
	:	:	:	:	:	:	:
0.0000	180	93	60	27		26	91
0.00232	222	227	162	172		98	185
0.003480	246	280	165	181		126	241
0.00464	261	305	205	190		159	269
0.00696	260	302	204	189		158	267
0.00928	259	304	203	187		157	268
0.01160	261	303	202	188		158	269
0.01392	262	302	203	187		159	267

TABLE XIV.

Growth of Yeast, Varying CaCl_2 .

25° C.			30° C.		35° C.		40° C.	
Normality: I = 1: I = 1: I = 1: I = 1: I = 1: I = 1: I = 1: I = 1								
of : Count:			: Count: Count: Count: Count		: Count: Count: Count		: Count	
CaCl_2 - : 24 hr: 48 hr: 72 hr: 48 hr: 42 hr: 98 hr: 24 hr: 48 hr								
0.000	61	92	217	186	157	202	160	210
0.0180	73	106	235	198				
0.0360	91	117	247	202	161	257	209	269
0.0540	105	129	289	246				
0.0720	130	136	295	266	177	260	212	272
0.0900	140	156	304	290				
0.108	158	173	312	298	191	274	226	285
0.126	219	196	310	310				
0.1440	256	218	318	318	211	278	245	299
0.1620	286	278	335	342	232	286	251	
0.1800	379	334	386	388	255	319	275	325
0.216	375	333	387	388	253	318	276	328
0.270	378	335	388	389	254	315	274	326
0.324	376	335	387	389	255	318	275	324
0.3600					254	317	276	

TABLE XV.

Growth of Yeast, Varying CaCO_3 .

	25° C.	30° C.	35° C.	40° C.
Normality of CaCO_3	I = 1 Count 32 hr.	I = 1 Count 48 hr.	I = 1 Count 36 hr.	I = 1 Count 46 hr.
0.000	42	130	65	70
0.0080	115	179	170	196
0.0120	125	298	192	210
0.0160	170	371	220	251
0.0200	100	238	180	215
0.0240	80	226	164	190
0.032	65	206	150	140
0.040	52	186	148	139
0.048	51	175	139	

VI. Growth Rates of Yeast.

Using a medium of uniform composition with plenty of food present, and plenty of room for the cells to grow, it is natural to expect that each cell would grow independently of the others, and that therefore the rate of increase of the number of cells in the flask at any moment would be proportional to the number of cells then present in the flask, i. e., (writing "C" for "Count"),

$$\frac{dC}{C} = kdt \quad \text{Integrating, we have}$$

$$\int_I^C \frac{dC}{C} = \int_{t_1}^{t_2} kdt$$

Therefore,

$$\log C - \log I = k(t_2 - t_1)$$

Letting $I = 1$,

$$\log C = kt$$

$$\text{then } k = \frac{\log C}{t}$$

During the logarithmic period of growth the plot of $\log C$ against t is a straight line.

Having determined the optimum medium for the growth of yeast at various temperatures the author proceeded to determine the growth rates for the various temperatures using the optimum medium for each particular temperature and from these results calculated the thermal coefficients. The same determinations were also made upon wort, and wort with the

first and second maxima concentrations of ammonium chloride.

1. The Growth Rates of Yeast in Wort and Wort plus Ammonium Chloride.

The results obtained with wort and wort plus ammonium chloride are shown in Tables 16, 17, 18, 19, and 20. Tables 16 and 17 give the count for the various time intervals for wort and also for the first and second maxima. Table 18 shows the critical count and maximum crop of yeast for each temperature studied. It is interesting to note that both the critical count and the maximum crop are increased by the addition of ammonium chloride. Table 19 gives the time in hours and the corresponding log, C, for wort and the first and second maxima at each temperature. From these values if log C is plotted as ordinate and time as abscissae we get a straight line during the logarithmic growth rate. Table 20 shows the optimum concentration of ammonium chloride for the first and second maxima, k and k_{10}/k or the thermal coefficient for each rise of 10 degrees in temperature.

TABLE XVI.

The Effect of Ammonium Chloride Upon the Growth Rates of Yeast in Wort.

		: 10 degrees :				: 20 degrees :	
		: First :	: Second :			: First :	: Second :
		: Max. :	: Max. :			: Max. :	: Max. :
Time	Count	: Count	: Count	Time	Count	: Count	: Count
		:	:			:	:
12	3.3	3.5	3.4	17	58	72	65
15	4.4	4.8	4.6	18	73	92	83
17	5.4	6.0	5.7	19	92	119	107
19	6.6	7.2	6.8	21	148	196	175
26	13.3	14.9	14.0	25	290	384	342
32	23.8	28.4	26	34	966	1159	1040
45	86	109	98	43	1085	1301	1180
50	102	153	133	55	1170	1404	1270
62	158	237	206	67	1218	1449	1296
74	160	244	210	75	1218	1444	300
81	161	246	212				

TABLE XVII.

The Effect of Ammonium Chloride Upon the Growth Rates of Yeast in Wort.

	:	:	:	:	:	:	:	:	:	:	:
	:	:First	:Second:	:	:	:First	:Second:	:	:	:First	:Second
	:Wort	:Max.	:Max.	:	:Wort	:Max.	:Max.	:	:Wort	:Max.	:Max.
Time	:Count	:Count	:Count	Time	:Count	:Count	:Count	Time	:Count	:Count	:Count
	:	:	:	:	:	:	:	:	:	:	:
8	22	29	25	10.3	99	131	113	9	54	64	59
10	48	56	54	11.3	159	209	178	11	131	161	146
11	74	87	82	12.3	242	332	277	12	206	255	228
12	104	130	122	15	810	1187	980	13	312	406	356
21.5	1245	1437	1300	25.5	950	1330	1200	23	450	600	525
34	1340	1470	1390	47	1365	1465	1380	35	504	652	617
58	1345	1510	1395	69	1550	1705	1565	48	612	667	655
70	1350	1515	1400	81	1555	1710	1560	59	620	670	662
								80	622	665	660

TABLE XVIII.

The Effect of Ammonium Chloride Upon the Growth of Yeast in Wort.

	Wort		: First Maximum		: Second Maximum	
	: Critical	: Maximum	: Critical	: Maximum	: Critical	: Maximum
Temperature	: Count	: Crop	: Count	: Crop	: Count	: Crop
10	102	161	153	246	133	212
20	290	1218	384	1442	342	1300
30	1245	1350	1437	1515	1300	1400
40	950	1555	1330	1710	1200	1560
42	450	622	600	665	525	660

TABLE XIX

The Effect of Ammonium Chloride Upon the Growth Rate of Yeast in Wort.

10				20				30			
:First:Second				:First:Second				:First:Second			
Time:Wort	Max.	Max.		Time:Wort	Max.	Max.		Time:Wort	Max.	Max.	
Hrs.:Log C:Log C:Log C				Hrs.:Log C:Log C:Log C				Hrs.:Log C:Log C:Log C			
12	0.5185	0.5490	0.5315	17	1.7634	1.8573	1.8129	8	1.3464	1.4683	1.3962
15	0.0435	0.6812	0.6628	18	1.8633	1.9638	1.9191	10	1.6839	1.7649	1.7396
17	0.7324	0.7782	0.7559	19	1.9638	2.0755	2.0294	11	1.8692	1.9395	1.9138
19	0.8195	0.8573	0.8325	21	2.1703	2.2923	2.2430	12	2.0171	2.1139	2.0864
26	1.1239	1.1734	1.1461	25	2.4624	2.5843	2.5340	21.5	2.0951	2.1574	2.1139
32	1.3766	1.4533	1.4150								
45	1.9345	2.0374	1.9934								
50	2.0086	2.1847	2.1239								
40 degrees				42 degrees							
10.3	1.9956	2.1139	2.0531	9	1.7324	1.8062	1.7709				
11.3	2.2014	2.3201	2.2520	11	2.1173	2.2041	2.1644				
12.3	2.3838	2.5239	2.4425	12	2.3139	2.4065	2.3579				
15	2.9085	3.0735	2.9880	13	2.4942	2.6085	2.5574				
25.5	2.9777	3.1239	3.0792	23	2.6532	2.7782	2.7202				

TABLE XX.

The Effect of Ammonium Chloride Upon the Growth Rate of Yeast in Wort.

Temperature	Wort			First Maximum			Second Maximum		
	Norm-	ality:		Norm-	ality:		Norm-	ality:	
	:NH ₄ Cl:	k	:k ₁₀ /k:	:NH ₄ Cl:	k	:k ₁₀ /k:	:NH ₄ Cl:	k	:k ₁₀ /k:
	:	:	:	:	:	:	:	:	:
10	0.00	0.0430		0.0416	0.0453		0.0649	0.0443	
20	0.00	0.1035	2.40	0.01888	0.1092	2.41	0.0826	0.1068	2.41
30	0.00	0.1686	1.62	0.0236	0.1780	1.63	0.1060	0.1740	1.63
40	0.00	0.1936	1.14	0.02832	0.2051	1.15	0.1180	0.1992	1.145
42	0.00	0.1923	0.99	0.0294	0.2005	0.977	0.1230	0.1964	0.972

It is a significant fact that although k has a higher value in the case of the two optima concentrations of ammonium chloride than in wort alone, the thermal coefficients in each case are the same. In other words, the percentage increase in the growth rate is apparently a constant.

2. Growth Rates of Yeast in Medium E.

The growth rates of yeast in medium E at various temperatures, using the optimum concentration of ammonium chloride for each temperature were determined and the thermal coefficients were calculated from these. Growth rates for normal wort were also obtained at the same time and the thermal coefficients calculated. The results are shown in Tables 21 to 25 inclusive. Tables 21, 22 and 23 give the time and the corresponding count for each temperature for wort and for Medium E, optimum for each temperature studied. Table 24 gives the critical count, maximum crop of yeast, k and k_{10}/k for each temperature.

It will be noticed that the best results were obtained at 40 degrees. At 40 degrees we obtain not only the highest critical count but also the highest maximum crop of yeast. This is of interest in view of the fact that most investigators have held to the view that yeast will not grow well at 40 degrees.

TABLE XXI.

The Effect of Ammonium Chloride Upon the Growth Rate of Yeast in Medium E.

0 degrees				10 degrees			
Time	Count	Count	Count	Time	Count	Count	Count
28	2.2	2.1	2.00	26	13.2	11.3	11.6
35	2.7	2.6	2.50	29.5	18.8	15.7	15.8
48	3.9	3.6	3.50	36	35.5	28.5	29
32	4.3	3.8	3.9	40	53	42	41.5
64	4.4	4.2	4.3	52	128	105	106
76	4.6	4.1	4.3	62	162	134	132.5
				77	164	137	134

TABLE XXII.

The Effect of Ammonium Chloride Upon the Growth of Yeast in Medium E.

20 degrees				30 degrees				40 degrees			
	Med.	Med.			Med.	Med.			Med.	Med.	
Wort	E(A)	E(B)		Wort	E(A)	E(B)		Wort	E(A)	E(B)	
Time	Count	Count	Count	Time	Count	Count	Count	Time	Count	Count	Count
17	57.3	34.2	32.7	7	15.5			6	4.5		
119	92.4	49.2	48.6	11	70.5	30	33	8	24.6		
21	149	74	73	12		45.5	45	9.5	67		
223	243	111	112	13	153			11		43.5	46
25	281	128	129	14	225	77	77	12	223		
29.5	792.5	149	150	15.25		110.5	107.5	12.5		63.5	61
39.5	1165	224	226	16		137	140	13	320		
48	1190	280	285	23	677			13.5		91.5	92.5
63.5	1205	283	281	29	1050			14	505		
				35.25	1092	156	164	14.5		112.5	113.5
				39		191	192	22		169	166
				40.5	1197			23.5	837.5		
				43.25		254	258	29	872.5		
				46.5	1205	261	265	34		223	228
				48	1220			37	1342		
				54		274	279	45		395	390
				59.25	1215			48	1345		
				65		270	272	58		393	392
				73	1223			60	1332		
								68		399	399
								72	1342	398	391

TABLE XXIII.

The Effect of Ammonium Chloride Upon the Growth Rate of Yeast in Medium B.

42 degrees				45 degrees			
Time	Count	Count	Count	Time	Count	Count	Count
9	53	13.3	13	12	1.6	1.5	1.5
10	83	17.2	18	18	2.2	1.8	1.8
11	131	22.7	22.5	24	2.8	2.2	2.1
13	308	41.5	41	30	3.6	2.6	2.7
15	316	70.5	72.5	38	3.7	3.3	3.4
17	340	131	129	45	3.8	3.4	3.5
21	355	162	159	68	3.8	3.5	3.4
33	408	258	250				
41.5	521	332	336				
56.5	625	349	343				
68.5	630	345	342				

TABLE XXIV.

The Effect of Ammonium Chloride Upon the Growth of Yeast in Medium E at Different Temperatures.

[illegible]

The Effect of Ammonium Chloride Upon the Growth Rate of Yeast
in Medium E.

Temperature:- 0 degrees				:	10 degrees			
Wort:		:Med.: (A) : (B)		:	Wort:		:Med.: (A) : (B)	
Time:Log.C.:Time:Log.C.:Log.C.:				:	Time:Log.C.:Time:Log.C.:Log.C.			
28	0.3424	28	0.3222 0.3010:	:	26	1.1205	26	1.0529 1.9645
35	0.4514	35	0.4150 0.3979:	:	29.5	1.2742	29.5	1.1959 1.1987
48	0.5911	48	0.5563 0.5441:	:	36	1.5502	36	1.4548 1.4624
52	0.6335	52	0.5798 0.5911:	:	40	1.7243	40	1.6232 1.6180
64	0.6435	64	0.6232 0.6335:	:	52	2.1072	52	2.0212 2.0253
20 degrees				:	30 degrees			
17	1.7597	17	1.5105 1.5132:	:	7	1.1903	11	1.4771 1.5185
19	1.9657	19	1.6920 1.6866:	:	11	1.8482	12	1.6580 1.6532
21	2.1732	21	1.8692 1.8633:	:	13	2.1847	14	1.8865 1.8865
23	2.3856	23	2.0453 2.0492:	:	14	2.3522	15.25	2.0433 2.0305
25	2.4487	25	2.1072 2.1106:	:	23	2.8309	16	2.1367 2.1461
40 degrees				:	42 degrees			
6	0.6532	11	1.6585 1.6628:	:	9	1.7243	9	1.1271 1.1139
8	1.3909	12.5	1.8028 1.7853:	:	10	1.9191	10	1.2340 1.2553
9.5	1.8325	13.5	1.9614 1.9661:	:	11	2.1239	11	1.5560 1.3522
12	2.3483	14.5	2.0511 2.0550:	:	13	2.4886	13	1.6180 1.6128
13	2.5051	22	2.2279 2.2201:	:	15	2.5079	15	1.8482 1.8693
14	2.7033	34	2.3483 2.3579:	:	17	2.5315	15	2.1173 2.1106
43 degrees				:				
12	0.2041	12	0.1761 0.1761:	:				
18	0.3424	18	0.2553 0.2780:	:				
24	0.4472	24	0.3424 0.3222:	:				
30	0.5563	30	0.4150 0.4314:	:				
38	0.5682	38	0.5185 0.5315:	:				

At 42 degrees the yeast are growing fairly well but at 43 degrees there is a very sharp break in the growth rate and also in the total crop of yeast. In fact the break is so sudden that it is quite difficult to obtain a very accurate growth rate.

Table 25 gives the time in hours and Log. C. for the corresponding time.

In Figure 2 the values of k for the various temperatures are plotted as ordinates and temperature as abscissa. Plotting k against temperature we obtain a straight line, except from 0 to 10 degrees where there is apparently a lag phase, and from 40 degrees on, where there is a gradual falling off until 42 degrees is reached while from 42 degrees to 43 degrees there is a sudden drop, k dropping to 0.0143 for Medium E and to 0.0180 for wort.

5. Growth Rates for Medium E Optimum for 30 degrees.

Growth rates were determined for Medium E using the optimum concentration of Ammonium Chloride for 30 degrees, at 10, 20, 30, 40 and 42 degrees. The results are shown in Tables 26 to 28 inclusive and Figure 2.

TABLE XXVI.

Growth Rates of Yeast in Medium E. (Optimum for 30 degrees).

10 deg.			20 deg.			30 deg.			40 deg.			42 deg.		
:E (A):E (B):			:E (A):E (B):			:E (A):E (B):			:E (A):E (B):			:E (A):E (B):		
Time	Count	Count	Time	Count	Count	Time	Count	Count	Time	Count	Count	Time	Count	Count
15	3	3.1	18	24	25	11	33	30	8	11.2	11.4	8	7.5	7.3
19	4.2	4.1	21	42	43	12	45	46	10	21	20	10	13.5	13.2
23	5.5	5.9	24	72	73	14	77	79	11	28	28.5	13	26.5	26
30	92	9.7	28	80	82	15.25	110	108	12	38	38.5	16	55	56
39	18.2	18	41	174	177	25.5	137	140	16	97	99	25	89	90
55	37	39	50	184	187	35.25	156	164	32	144	141	39	132	133
64	67	70	65	205	207	39	192	191	42	188	183	51.5	139	137
72	69	73	75	203	210	43.25	258	254	56	195	198	63	138	138
						48	261	265	69	196	201			
						59.25	274	279	80	202	206			
						71	270	272						

TABLE XXVII.

The Effect of Ammonium Chloride Upon the Growth of Yeast in Medium B.
(Optimum for 30 deg.).

10 deg.			20 deg.			30 deg.			40 deg.			42 deg.		
Time	Log. C	Log. C	Time	Log. C	Log. C	Time	Log. C	Log. C	Time	Log. C	Log. C	Time	Log. C	Log. C
15	0.4771	0.4914	18	1.3909	1.3979	11	1.4771	1.5185	8	1.0492	1.0569	8	0.8751	0.8633
19	0.6232	0.6128	19	1.4744	1.4771	12	1.6580	1.6532	10	1.3222	1.3118	10	1.1303	1.1206
23	0.7404	0.7709	21	1.6274	1.6335	14	1.8865	1.8865	11	1.4472	1.4548	13	1.4232	1.4150
30	0.9638	0.9868	22	1.7050	1.7076 ^{15.35}	2.0433	2.0305	12	1.5798	1.5855	16	1.7404	1.7482	
39	1.2691	1.2553	24	1.8573	1.8637	16	2.1367	2.2461	16	1.9868	1.9956	25	1.9494	1.9542
55	1.5740	1.5911	28	1.9031	1.9138									

TABLE XXVIII.

The Effect of Ammonium Chloride Upon the Growth Rate of Yeast in Medium E. at Various Temperatures Using a Medium Optimum for 30 degrees C.

E - I			E - II				
	:Critical:	:Maximum:		:Critical:	:Maximum:		
Temperature:	Count	:Crop	Count	:Crop	: k	:k ₁₀ /k	
10	37	69	39	73	0.0324		
20	80	203	82	210	0.0777	2.398	
30	156	270	107	272	0.1361	1.751	
40	97	202	99	206	0.1316	0.966	
42	89	138	90	139	0.1098	0.834	

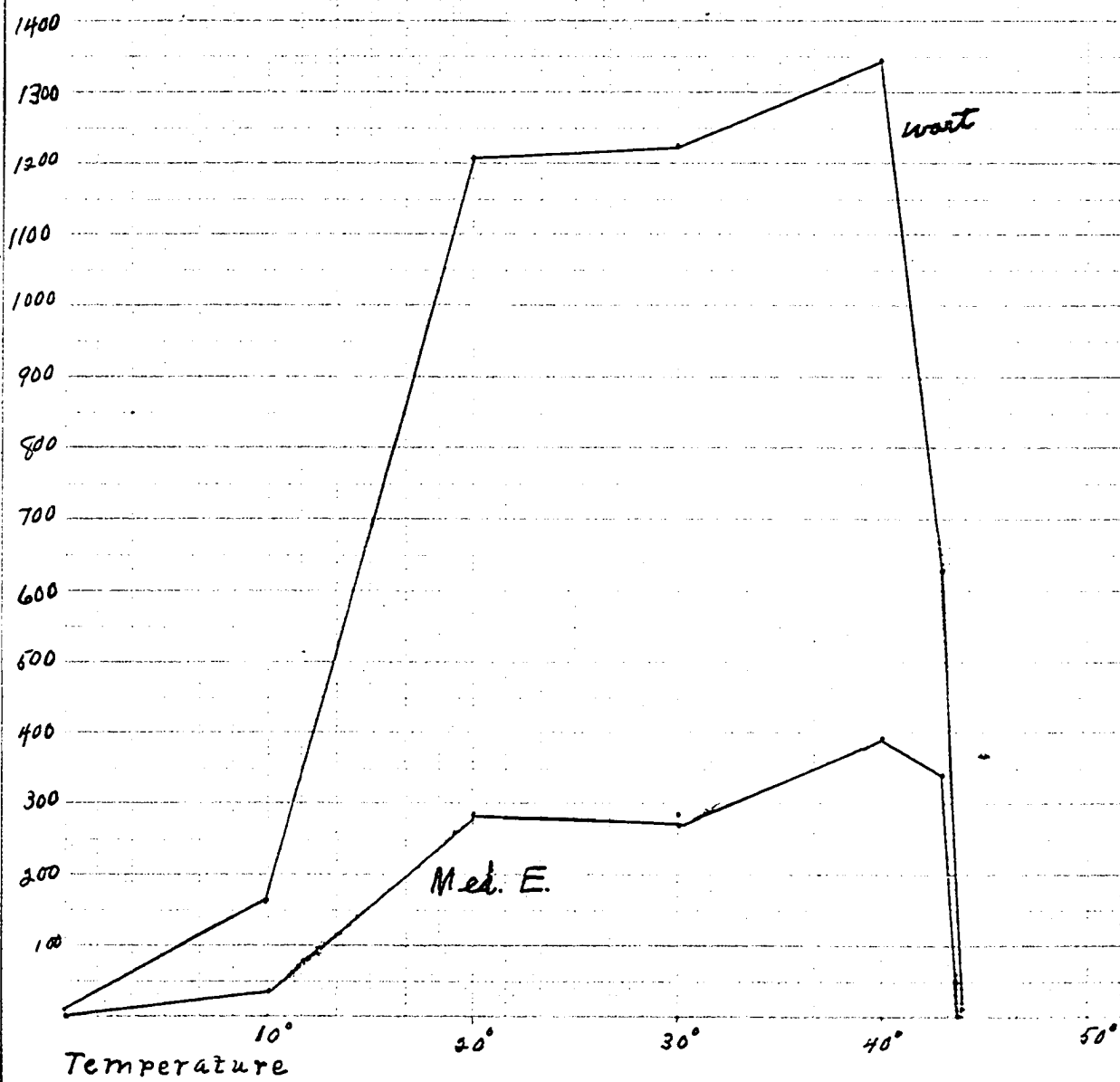


Table 26 shows the time and the count for the temperatures studied while table 27 gives the time and log. C. for the corresponding temperatures. Table 28 gives the critical count, the maximum crop, and k and k_{10}/k for the various temperatures studied.

It will be noticed in Figure 2 that k for Medium E (Optimum for 30 degrees) is below the corresponding k for the optimum medium for that particular temperature; for example at 10 degrees the value of k for the optimum medium E. is 0.0405 while the value for k at 10 degrees using a medium E. optimum for 30 degrees gives a lower value, namely 0.0324. The same is true for 20 degrees while at 30 degrees, where the composition of the medium is optimum, k is on the curve plotted for media optimum for each temperature. However at 40 degrees the value of k shows a very decided drop again, k dropping from 0.1452 to 0.1316 while at 42 degrees it drops from 0.1239 to 0.1098. It is evident from a study of Figure 2 that the values of k (using a medium optimum for 30 degrees) at 10 degrees and 30 degrees are too low and again above 30 degrees they are too low, while the value for k or k_{10}/k are too high for temperatures below 30 degrees and too low for values above 40 degrees, as shown by a comparison of Tables 24 and 28. The explanation for this is that as we increase the temperature we are also approaching the optimum temperature for the medium used, so that we have two factors tending to increase the growth rate,

Fig. 2.

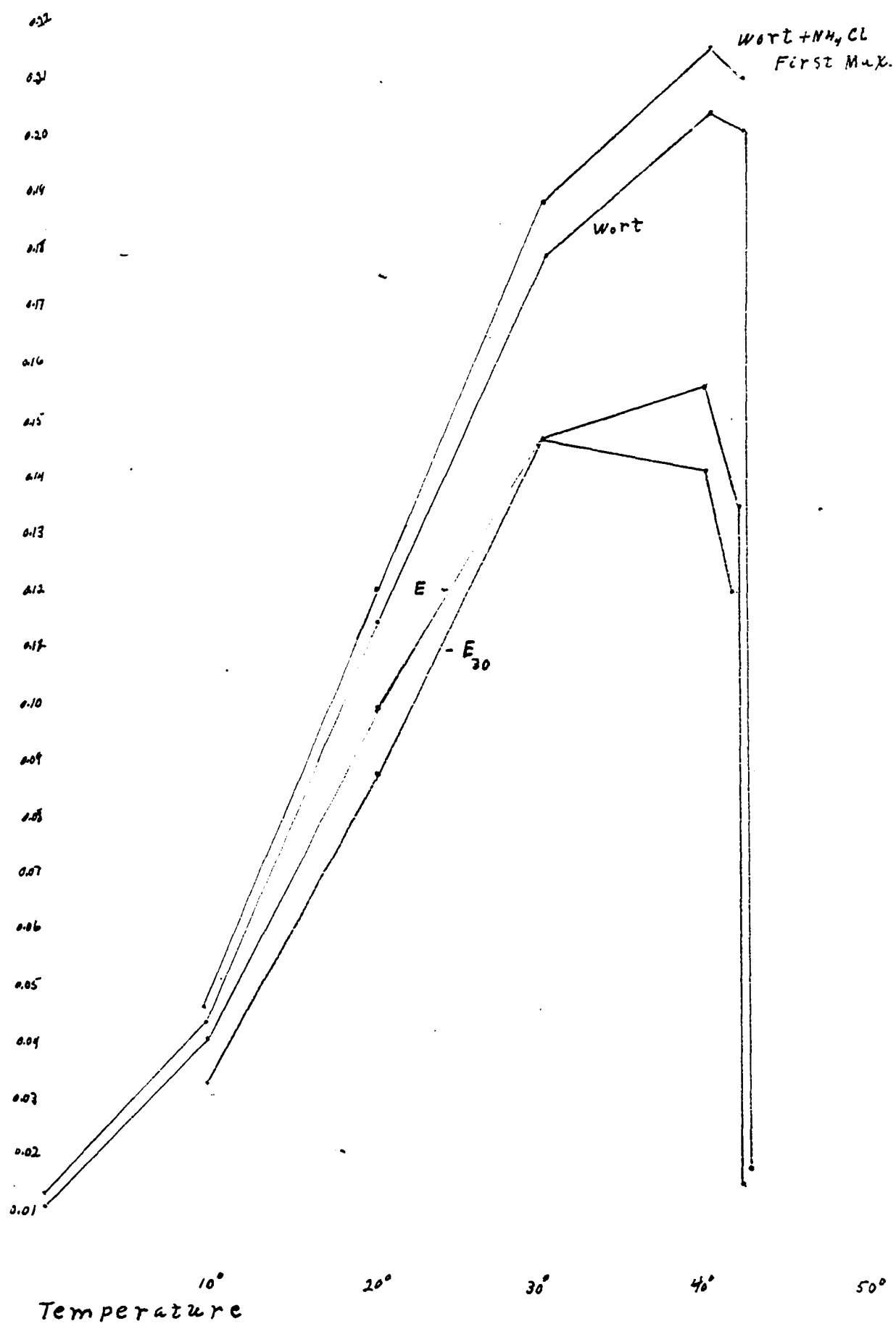
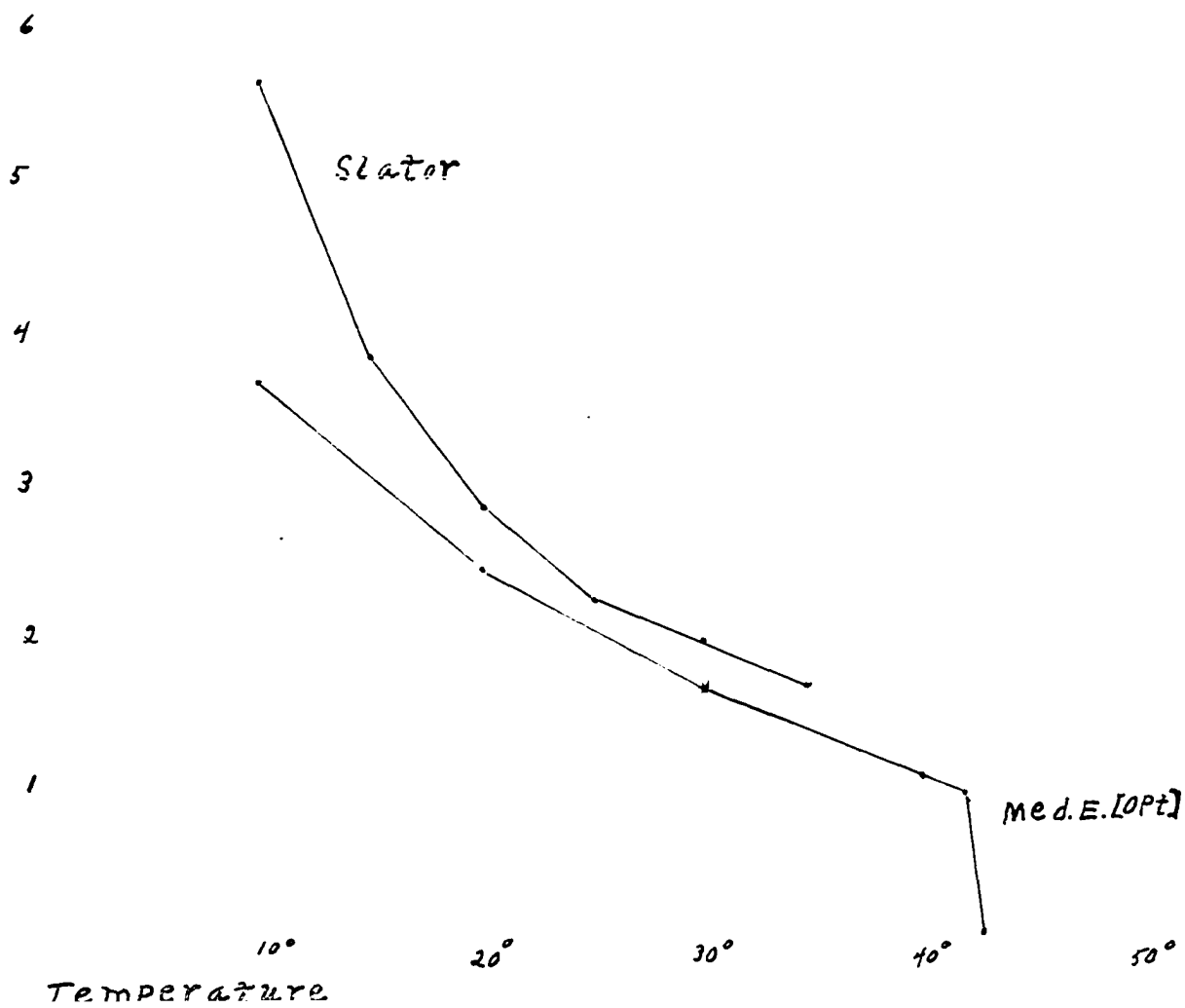


TABLE XXIX.

Slator		Medium E.	
k_{10}/k Experiment	k_{10}/k Calculated	k_{10}/k Experiment	k_{10}/k Calculated
5.6	2.162	3.521	1.770
2.25	2.017	2.180	1.65
1.60	1.886	1.541	1.541
		1.066	1.443



rise in temperature and the change in the thermodynamic environment until the optimum temperature for the given medium is reached, while from there on up these two factors work against each other, that is, a rise in temperature will tend to increase the growth rate, while the change in the thermodynamic environment of the medium will tend to decrease the growth rate, hence the values for k_{10}/k will fall below the value of k_{10}/k where physico-chemical conditions are optimum.

Table 29 gives the thermal coefficients for 10 degree intervals for the growth of yeast from Slator's work and those obtained from Medium E., together with the calculated values (using formula (20), page 14) in each case. The experimental values do not agree very closely with the calculated values. However they do agree somewhat more closely in the case of Medium E. than they do with Slator's figures.

These experiments were carried out in the Bio-Physical Chemistry Laboratory of the Iowa State College under the direction of Dr. E. I. Fulmer.

VII. Summary.

1. The method given in a former paper⁵¹ by which the optimum concentration of each of the constituents of the medium for the growth of yeast has been extended to include other temperatures. The results confirm the former findings.
2. The effect of ammonium chloride upon the growth of yeast in wort was studied. Two maxima concentrations of ammonium chloride were found which varied with the temperature.
3. A second maximum concentration of ammonium chloride was also found for the growth of yeast in Medium E.
4. Thermal coefficients for the growth of yeast in various media were determined in similar physico-chemical environments for each temperature.
5. The average K_{10}/K for four investigators was 2.72 to 2.88. Determined as in 4, the average was 2.05, showing a material difference in methods.

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